
सूक्ष्मजीव जीवाणु कल्चर का समूह
(मिश्रित जैवउर्वरक जीवाणु कल्चर) —
विशिष्टि

Consortia of Microbial Inoculants
(Mixed Biofertilizer Inoculants) —
Specification

ICS 65.080

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भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुरशाह ज़फर मार्ग, नई दिल्ली – 110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002
www.bis.gov.in www.standardsbis.in

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soil Quality and Fertilizers Sectional Committee had been approved by the Food and Agriculture Division Council

Bio fertilizers have been identified as alternative to chemical fertilizers to increase soil fertility and crop production in sustainable farming. Bio fertilizers are natural fertilizers which are microbial inoculants of bacteria, algae, fungi alone, or in combination, and they augment the availability of nutrients to the plants. The use of bio fertilizers, in preference to chemical fertilizers, offers economic and ecological benefits by way of soil health and fertility to farmers.

Biofertilizers are products containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available form through biological processes. They are involved in symbiotic and associative microbial activities with higher plants. These are natural mini-fertilizer factories that are economical and safer source of plant nutrition for increasing the agricultural production and improving soil fertility.

Since the development and commercialization of phosphate solubilizing bacterial and fungal inoculants, voluminous data has been generated on the synergistic effects of these micro-organisms when they are applied together. Now it has been established beyond doubt that combined application of nitrogen fixing, phosphate solubilizing and growth promoting bacteria delivers best results. Since last many years, combined application of at least one nitrogen fixing and phosphate solubilizing bacteria is being promoted and recommended. Keeping in view the advantages of combined application, various industries have developed their own mixed inoculants combining either two (one N-fixer + PSBI), three (one N-fixer + PSBI + KMBI) or four bio fertilizer organisms (one or two N-fixer + PSBI + KMBI + *Pseudomonas fluorescens*).

The Consortia of Microbial Inoculants (CMI) include different groups of beneficial soil micro-organisms, such as nitrogen fixers, phosphate solubilizers, potash mobilizers, zinc solubilizers and plant growth promoters which harvest the nutrients in unavailable form from the air and soil and convert them into plant usable form. Some bacteria secrete growth promoting hormones and help in plant growth and root development through their hormonal action. The species of *Rhizobium*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Fraturia*, *Gluconacetobacter*, *Pseudomonas fluorescens* etc. are active in such bioconversion. These efficient micro-organisms have shown capability to mobilize nitrogen, phosphorus, potassium and zinc content available in the soil as natural reserve or supplied through fertilizer application in the soil.

Many brands of CMI are marketed in the country and they have been found to vary in quality. Hence, the need was felt for an Indian Standard not only to test the quality of inoculants in order to provide the farmers with certified inoculants but also to help the producers to improve quality of their products.

The composition of the Committee responsible for the formulation of this standard is given at Annex E.

It is obligatory on the part of the manufacturers to employ qualified soil microbiologist or microbiologist or agricultural graduates or graduates in biology trained in soil microbiology in their staff. Manufacturers shall also maintain a quality control laboratory capable of carrying out the tests according to this standard.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the result of a test or analysis shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (revised)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

CONSORTIA OF MICROBIAL INOCULANTS (MIXED BIOFERTILIZER INOCULANTS) — SPECIFICATION

1 SCOPE

The standard prescribes the requirements, method of sampling and tests for Consortia of Microbial Inoculants (CMI).

2 REFERENCE

The following Indian Standards contain provisions which through reference in this text constitutes provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below:

<i>IS No.</i>	<i>Title</i>
460 (Part 1): 2020	Test sieves— Specification Part 1 Wire cloth test sieves (<i>fourth revision</i>)
1070 : 1992	Reagent grade water (<i>third revision</i>)
6850 : 1973	Specification for agar, microbiological grade
6853 : 1973	Specification for peptone, microbiological grade
7004 : 1973	Specification for yeast extract, microbiological grade
8268 : 2020	<i>Rhizobium</i> inoculants — Specification (<i>third revision</i>)
9138 : 2020	<i>Azotobacter</i> Spp. Inoculants — Specification (<i>third revision</i>)
14806 : 2000	<i>Azospirillum</i> inoculants
14807 : 2021	Phosphate solubilising bacterial inoculant (PSBI) — Specification (<i>first revision</i>)
17672 : 2021	Potash Mobilizing Bacterial Inoculant (KMBI) — Specification

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1.1 Consortia of Microbial Inoculants (CMI)

CMI is a product having high population of two or more beneficial plant growth promoting microorganisms and their strain(s) intended to improve plant growth and productivity through the processes of nitrogen fixation, nutrient solubilization or mobilization and through plant growth promotion.

3.1.2 Group of Crops

CMIs can be crop specific or of wide spectrum in nature for use in all types of crops for nutrient mobilization and growth promotion.

4 REQUIREMENTS

4.1 CMI shall be carrier based, colour depending on the colour of carrier. Carrier material, such as peat, lignite, charcoal or similar material may be used. In case carrier is acidic in nature, then it shall be neutralized with calcium carbonate and then sterilized. When tested for fineness, it shall pass through 0.15-0.212 mm IS sieve [(see IS 460 (Part 1)].

4.2 When tested by the method prescribed in Annex A of this standard, CMI shall contain a minimum of 1×10^6 viable cells of each of the constituent microorganism individually, per gram of the carrier material on dry mass basis during the entire period of shelf life, as specified on the packet.

4.3 When tested by the method prescribed in Annex A, CMI shall have no contamination with other micro-organisms at 10^5 dilution.

4.4 When tested by the method prescribed in Annex B of IS 8268, the pH of CMI shall be 5.0 to 7.5.

4.5 Individual microorganism present in CMI as per label claim, shall demonstrate minimum bio efficacy value as follows:

<i>Rhizobium</i>	effective nodulation on all the legume species listed on the pack
<i>Azotobacter</i>	10 mg N fixation/g of C-source utilized
<i>Azospirillum</i>	10 mg of N-fixation/g of malate applied

Phosphate Solubilizing Bacterial Inoculant (PSBI) — Specification (first Revision) 10 mm zone of solubilization on PSBI media having at least 3 mm thickness

Potash Mobilizing Bacterial Inoculant (KMBI) — Specification 10 mm zone of solubilization on KMBI media having at least 3 mm thickness

Zinc Solubilizing Bacterial Inoculant (ZnSBI) Specification 10 mm zone of solubilization on ZnSBI media having at least 3 mm thickness

Pseudomonas Indole positive reaction as per the method prescribed in Annex B

fluorescens

The bio efficacy value for *Rhizobium* inoculants shall be tested as described in IS 8268; for *Azotobacter* as per IS 9138 and *Azospirillum* as per IS 14806, for PSBI as per IS 14807; for KMBI as per IS 17672 and for *Pseudomonas fluorescens* as per method prescribed in Annex B of this standard.

4.6 Specified mother culture shall be obtained from any recognized institution maintaining the mother culture. The manufacturer may control the quality of broth as given in Annex C, and get it verified at least by two institutions as mentioned below.

NOTE — At present National Centre of Organic Farming (NCOF), Ghaziabad and its six Regional Centres located at Bangalore, Bhubaneshwar, Hissar, Imphal, Jabalpur and Nagpur; Indian Agricultural Research Institute (IARI), New Delhi; Tamil Nadu Agricultural University (TNAU), Coimbatore; University of Agricultural Science, Bengaluru are sources for supplying the mother culture.

5 PACKING, MARKING AND STORAGE

5.1 Packing

CMI shall be packed in packaging material of low density polyethylene or polypropylene bags thickness of which shall not be less than 75 micron or in suitable HDPE or polypropylene or plastic or glass bottles.

5.2 Marking

5.2.1 The following particulars shall be legibly and indelibly marked on package:

- Name of the product specially as 'CONSORTIA OF MICROBIAL INOCULANT';
- Name and address of the manufacturer;

- Crop(s) for which intended;
- Type of the carrier;
- Batch number;
- Date of manufacture;
- Expiry date which shall not be less than 6 months from the date of manufacture;
- Net quantity in kg and area meant for;
- Storage instructions worded as under "STORE IN COOL PLACE AWAY FROM DIRECT SUNLIGHT AND HEAT"; and
- Any other information required under the *Legal Metrology (Packaged Commodities) Rules*, 2011.

5.2.2 Items (c), (f) and (g) shall be printed on a coloured ink background.

5.2.3 Directions for use of CMI shall be printed briefly on the packets as given in Annex D of this standard. A separate pamphlet may preferably be given with it.

5.2.4 BIS Certification Marking

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

5.3 Storage

CMI shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 20 °C and not exceeding 30 °C. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.

6 SAMPLING

The representative samples of the CMI shall be drawn as prescribed in Annex F of IS 8268.

7 TESTS

7.1 Tests shall be carried out by the methods prescribed in 4.

7.2 Unless otherwise specified, quality reagents, chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect experimental results.

ANNEX A

(Clause 4.2 and 4.3)

DETERMINATION OF NUMBER OF INDIVIDUAL MICROBIAL CELLS

A-1 APPARATUS

A-1.1 Pipettes, graduated, 1 ml and 10 ml.

A- 1.2 Conical Flasks, 150 ml and 250 ml.

A- 1.3 Screw Capped Tubes, 10 ml.

A-1.4 Incubator

A-1.5 Petri Dishes

A-1.6 Hot Air Oven

A-1.7 Autoclave

A-1.8 pH Meter

A-2 REAGENTS

A-2.1 Use a medium of following compositions for different microorganisms:

A-2.1.1 Medium for Enumeration of Rhizobium

Agar (<i>see</i> 6850)	20 g
Yeast extract (<i>see</i> 7004)	1 g
Mannitol	10 g
Potassium hydrogen phosphate (K_2HPO_4)	0.5 g
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Sodium chloride (NaCl)	0.1 g
Congo red	2.5 ml
Distilled water	1 000 ml
pH	7.0

A-2.1.2 Medium for Enumeration of Azotobacter

Agar	20 g
Sucrose ($C_{12}H_{22}O_{11}$)	20 g
Ferric sulphate $Fe_2(SO_4)_3$	0.1 g
Dibasic potassium phosphate (K_2HPO_4)	1.0 g
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.5 g
Sodium chloride (NaCl)	0.5 g
Calcium carbonate ($CaCO_3$)	2.0 g
Sodium molybdate (Na_2MoO_4)	0.005 g
Distilled water	1 000 ml
pH	6.8 to 7.2

A-2.1.3 Medium for Enumeration of Azospirillum (Nitrogen Free Bromothymol Blue Medium)

Malic acid	5.0 g
Potassium hydroxide	4.0 g
Di-potassium hydrogen phosphate	0.5 g
Ferrous sulphate	0.05 g
Manganese sulphate	0.01 g
Magnesium sulphate	0.1 g
Sodium chloride	0.2 g
Calcium chloride	0.1 g
Sodium molybdate	0.002 g
Distilled water	1 000 ml
Bromothymol blue	2.0 ml
(0.5 Percent alcoholic solution)	
Agar	1.7 g
pH adjusted to	6.5 - 7.0

NOTE — Colour of the medium should be pale green.

A-2.1.4 Medium for Enumeration of Phosphate Solubilizing Microorganisms

Glucose	10.0 g
Tri-calcium phosphate	5.0 g
Ammonium sulphate	0.5 g
Magnesium sulphate	0.1 g
Sodium chloride	0.2 g
Yeast extract	0.5 g
Manganese sulphate	Trace
Ferrous sulphate	Trace
Distilled water	1 000 ml
Agar	15.0 g
pH adjusted to	7 ± 0.2

A-2.1.5 Medium for Enumeration of Potassium Mobilizing Microorganisms (Aleksandrov Medium)

Glucose	5g
Magnesium sulphate	0.005g
Ferric chloride	0.1 g
Calcium carbonate	2.0 g

Potassium alumino silicate (mica powder)	2.0g	K ₂ HPO ₄	1.5 g
Calcium phosphate	2.0 g	MgSO ₄ ·7H ₂ O	1.5 g
Distilled water	1 000 ml	Agar	15 g
Mix all the ingredients in about 750 ml of water, except glucose and autoclave. Filter sterilized glucose dissolved in 250 ml water is mixed with above solution after autoclaving.		Distilled water	1 000 ml
		pH adjusted to	7.2

A-2.1.6 Medium for Enumeration of Zinc Solubilizing Microorganisms

Glucose	10 g
Zinc oxide	1.0 g
Ammonium sulphate	0.5 g
Potassium chloride	0.2 g
Yeast extract	0.5 g
Ferrous sulphate	0.01 g
Manganese sulphate	0.01 g
Dipotassium hydrogen phosphate	0.5 g
Distilled water	1 000 ml

A-2.1.7 Medium for Enumeration of *Pseudomonas Fluorescens* (King's B Medium)

Proteose peptone (see 6853)	20 g
Glycerol	10 g

A-2.3 Sterilization and preparation process for plates is the same as described in IS 8268.

A-3 PREPARATION OF SERIAL DILUTION FOR CELL COUNT METHOD

Dispense 30 g of CMI in 270 ml of sterile water and shake for 10 minutes on a reciprocal shaker. Make serial dilutions up to 10⁷ level. Pipette out 0.2 ml aliquots of 10⁵ to 10⁷ dilution and deliver it on the petri dishes containing set medium as described in **A-2.1** on respective medium of each microorganisms to be enumerated, except *Azospirillum*. Spread the aliquots over the plate. Invert the plates and place them in the incubator at 28 ± 2 °C for 7 days. Use 3 replicates of 10⁵, 10⁶ and 10⁷ dilution. For *Azospirillum*, use the methods as described in IS 14806.

A-4 COUNTING

Count total number of colonies on the plates/tubes as per the method described for each microorganism.

ANNEX B

(Clause 4.5)

METHODS FOR ENUMERATION OF BIOEFFICACY CHARACTERISTICS OF
*PSEUDOMONAS FLUORESCENS***B-1 METHOD FOR INDOLE REACTION ASSAY****B-1.1 Medium for Growing Bacteria**

Add 0.25 g of Tryptophan into 1 litre of Kings B medium as given in A-2.1.7.

B-1.2 Growing Bacteria in Specified Medium

Fill 125 ml medium in six conical flasks and autoclave at 121°C for 15 min. After cooling, inoculate 3 flasks with loopful of bacteria (from a colony of *Pseudomonas fluorescens* grown on Kings B medium) and incubate for 5 days under continuous shaking at 28±2°C. Keep 3 uninoculated flasks as control. After 5 days of incubation, centrifuge the broth at 15 000 rpm for 30 min and collect the supernatant. Use this clear centrifuged solution for indole reaction.

B-1.3 Reagents

B-1.3.1 Stock Solution of 0.5M Ferric Chloride ($FeCl_3$)

B-1.3.2 Stock Solution of 35 Percent Perchloric Acid ($HClO_4$)

B-1.3.3 Orthophosphoric Acid

B-1.4 Procedure

B-1.4.1 Preparation of Salkowski Reagent — Suspend 1 ml of 0.5M $FeCl_3$ in 50 ml of $HClO_4$ (prepare fresh every time).

B-1.4.2 Take 2 ml of centrifuged broth in a test tube, add 2-3 drops of orthophosphoric acid followed by 4 ml of freshly prepared Salkowski reagent.

B-1.4.3 Incubate the tubes at room temperature for 25 min and observe the colour. Development of pink colour in cultured broth is indicative of indole positive reaction. Uninoculated broth (control) shall show no colour development.

ANNEX C

(Clause 4.6)

GUIDELINES ON MAINTENANCE AND PREPERATION OF CULTURE
AND QUALITY CONTROL AT BROTH STAGE**C-1 MAINTENANCE OF PURE CULTURES**

C-1.1 Maintain pure cultures of CMI on the medium as described in A-2.1 in the form of slants.

C-1.2 Transfer a loopful of pure culture to each of the agar slants aseptically in an inoculation room and incubate at 28 ± 2 °C for 7 days. Always keep pure culture below 5 °C.

C-2 PREPARATION OF INOCULUM CULTURE AND MASS CULTURE

Inoculum culture and mass culture shall be prepared as described in D-2 and D-3 of the IS 8268 changing the media composition as mentioned in A-2.1 of this standard.

C-3 QUALITY CONTROL TEST RECOMMENDED AT BROTH STAGE**C-3.1 Qualitative Test**

C-3.1.1 Check for freedom from visible contaminants by microscope and observing solubilization zones.

C-3.1.2 pH of bacterial broth shall normally be between 6.5 to 7.

C-3.1.3 Gram staining test shall be carried out as described in D-4.1.3.1 and D-4.1.3.2 of IS 8268.

C-3.2 Quantitative Test

C-3.2.1 Viable Cell Count — As per the method mentioned at Annex A of this standard.

ANNEX D

(Clause 5.2.3)

DIRECTION FOR USE OF CONSORTIA OF MICROBIAL INOCULANTS

D-1 The contents of the packet are sufficient enough to the given quantity of seed for seed treatment or for given area to be broadcast or for given quantity of seedlings for root dipping depending on the specified crops as denoted on the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. Dry the inoculated seed under shade over clean dry paper or organic bag and sow immediately. For seedling treatment, suspend recommended quantity of inoculants in recommended quantity of water and keep the roots of seedlings dipped

for 30-45 min immediately before transplanting. For soil treatment, mix recommended quantity of inoculants in 200-500 kg of mature compost and broadcast evenly over recommended area at the time of final ploughing before sowing.

D-2 In order to get best benefits, use CMI in crops specified on the packet. Use before the expiry date and do not expose to direct sunlight or heat.

D-3 CMI is not a chemical fertilizer, hence do not mix inoculated seeds or CMI with agro-chemicals.

ANNEX E*(Foreword)***COMMITTEE COMPOSITION**

Soil Quality and Fertilizers Sectional committee, FAD 07

<i>Organization</i>	<i>Representative(s)</i>
Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya	DR A. K. SINGH (Chairman)
Bidhan Chandra Krishi Vishwavidyalaya, West Bengal	PROF B. MANDAL PROF G. C. HAZRA (<i>Alternate</i>)
CCS Haryana Agricultural University, Hissar	HEAD (SOIL SCIENCE) HEAD (MICROBIOLOGY) (<i>Alternate</i>)
Central Fertilizers Quality Control and Training Institute, Faridabad	SHRI SHYAM BABU DIRECTOR
Chambal Fertilizers and Chemicals Ltd, New Delhi	DR NARESH PRASAD DR A. K. BARANWAL (<i>Alternate</i>)
Consumer Guidance Society of India, Mumbai	DR SITARAM DIXIT
Fertilizer Association of India, New Delhi	DR R. K. TEWATIA
G.B. Pant Univ. of Agri. and Technology, Pantnagar	DR ANIL K. SHARMA PROFESSOR
Indian Micro Fertilizers Manufacturers Association, Pune	SHRI MAHESH G. SHETTRY
Indian Agricultural Research Institute, New Delhi	HEAD (SOIL SCIENCE) HEAD (MICROBIOLOGY) (<i>Alternate</i>)
Indian Farmer Fertilizer Co-operative Limited (IFFCO), New Delhi	
Indian Institute of Soil Science, Bhopal	DR ASHOK K. PATRA DR M. C. MANNA (<i>Alternate</i>)
International Panacea Ltd, New Delhi	DR RANVIR SINGH MRS VIMALA PRAKASH (<i>Alternate</i>)
Ministry of Agriculture, New Delhi	ADDITIONAL COMMISSIONER (INM) DEPUTY COMMISSIONER (INM 1) (<i>Alternate</i>)
Ministry of Chemicals and Fertilizers, Dept of Fertilizers, New Delhi	SHRI BALVINDER KUMAR SHRI M. G. BANGA (<i>Alternate</i>)
National Bureau of Soil Survey and Land Use Planning, Nagpur	DR DIPAK SARKAR
National Centre of Organic Farming, Ghaziabad	SHRI V. K. BANSAL
National Fertilizer Ltd, Noida	DR JYOTI GOEL SHRI PREMLAL (<i>Alternate</i>)
NEERI, Nagpur	DR HEMANT J. PUROHIT DR B. K. SARANGI (<i>Alternate</i>)
Potash Research Institute of India, Gurugram	SHRI S. K. BANSAL
Punjab Agricultural University, Ludhiana	HEAD (SOIL SCIENCE) HEAD (SOIL SCIENCE) (<i>Alternate</i>)
Tamil Nadu Agricultural University, Coimbatore	DR R SANTHI
The Energy and Resources Institute, New Delhi	DR ALOK ADHOLEYA DR REENA SINGH (<i>Alternate</i>)

<i>Organization</i>	<i>Representative(s)</i>
University of Agricultural Sciences, Bengaluru	DR K. SUDHIR DR V. R. RAMAKRISHNA PARAMA (<i>Alternate</i>)
National Bureau of Agriculturally Important Microorganism(NBAIM)-Mau Nath Bhanjan (UP)	DR ANIL KUMAR SAXENA ALOK KUMAR SRIVASTAVA (<i>Alternate</i>)
Gujrat State Fertilizer & Chemicals Ltd-Vadodara (Gujrat)	MR V. N. GOTH, DY MANAGER (AS) MR K. G. KORANT, SR MANAGER (AS) (<i>Alternate</i>)
BIS Directorate General	MS SUNEETI TOTEJA, SCIENTIST 'E' AND HEAD (FAD) [REPRESENTING DIRECTOR GENERAL (<i>Ex-officio</i>)]

Member Secretary

SHRI RAJPAL
SCIENTIST 'D' (FAD), BIS

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BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002

Telephones: 2323 0131, 2323 3375, 2323 9402

Website: www.bis.gov.in

Regional Offices:

	Telephones
Central : 601/A, Konnectus Tower-1, 6 th Floor, DMRC Building, Bhavbhuti Marg, New Delhi 110002	{ 2323 7617
Eastern : 8 th Floor, Plot No 7/7 & 7/8, CP Block, Sector V, Salt Lake, Kolkata, West Bengal 700091	{ 2367 0012 2320 9474
Northern : Plot No. 4-A, Sector 27-B, Madhya Marg Chandigarh 160019	{ 265 9930
Southern : C.I.T. Campus, IV Cross Road, Taramani, Chennai 600113	{ 2254 1442 2254 1216
Western : Plot No. E-9, Road No.-8, MIDC, Andheri (East), Mumbai 400093	{ 2821 8093

Branches : AHMEDABAD. BENGALURU. BHOPAL. BHUBANESHWAR. CHANDIGARH. CHENNAI. COIMBATORE. DEHRADUN. DELHI. FARIDABAD. GHAZIABAD. GUWAHATI. HIMACHAL PRADESH. HUBLI. HYDERABAD. JAIPUR. JAMMU & KASHMIR. JAMSHEDPUR. KOCHI. KOLKATA. LUCKNOW. MADURAI. MUMBAI. NAGPUR. NOIDA. PANIPAT. PATNA. PUNE. RAIPUR. RAJKOT. SURAT. VISAKHAPATNAM.